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Determination of Manganese, Copper and Zinc in Serum and Packed Blood Cells by Neutron Activation Analysis

Studies on the metabolism of trace elements

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A method for simultaneous determination of manganese, copper and zinc in serum and packed blood cells by neutron activation analysis is described. After irradiation the samples are wet-ashed. Copper is extracted at pH 2, manganese and zinc at pH 8 with 0.05 mol/l oxine in chloroform. Radioactive measurements are carried out with a 3″ × 3″ NaI (Tl) well type detector coupled to a 400 channel analyser. Results are reported from 20 individuals.

Es wird eine Methode zur simultanen Bestimmung von Mangan, Kupfer und Zink in Serum und gepackten Blutzellen (Blutkuchen) mit der Neutronen-Aktivierungs-Analyse beschrieben. Nach Bestrahlung werden die Proben feucht verascht. Kupfer wird bei pH 2, Mangan und Zink werden bei pH 8 mit 0,05 mol/l Oxin in Chloroform extrahiert. Radioaktivitäts-Messungen werden mit einem 3″ × 3″ NaJ (Tl)-Detektor, verbunden mit einem 400-Kanal-Analyzer, durchgeführt. Die Ergebnisse von Bestimmungen bei 20 Probanden werden mitgeteilt.

Manganese, copper and zinc are essential trace elements in man (1—4). Several aspects of their metabolic functions (5—10) and their importance in pathology (11—14) have already been established.

Most published values for serum copper and zinc concentration show good agreement but those for manganese vary over a wide range (15—21).

We developed a sampling and analytical technique which reduces contamination to a minimum.

As it seemed desirable to obtain data concerning the intracellular level, determinations were also made on packed blood cells (blood clot).

Materials and Methods

Collection of samples

Quartz tubes (⌀ = 16.6 mm, h = 12 cm) for the collection of blood are cleaned with distilled water from a quartz apparatus and then boiled in a mixture of equal volumes of suprapur nitric and sulfuric acid for two periods of 2 h. They are rinsed again with triple distilled water, steamed for 3 h, dried and covered with thoroughly cleaned teflon stoppers until used. All operations are carried out in a "dust-free" room.

Blood samples for trace element analysis are collected after the disposable steel needle has been flushed by approximately 60 ml of blood. It has been demonstrated that even under these conditions the normal serum value for manganese can be increased by up to 10% by contamination, whereas the contaminations of copper and zinc are negligible (22).

The samples are placed in a water bath at 37°C for 3 h. Spontaneous clotting is allowed. Serum and blood clot are separated by centrifugation at 2500 rpm for 1 h and lyophilized.

Obviously, under these conditions, the separation of cellular elements and serum is incomplete. As was shown with albumin —^{131}I as tracer, ≦ 6% of the total extracellular fluid of whole blood is retained in the clot. This results in a negative error of about 7% for the measured intracellular manganese and zinc level and an approximate 5% positive error for the intracellular copper level. The reported values are not corrected for this systematic error.

After lyophilisation about 100 mg of dried serum or about 50 mg of dried packed cells are collected in thoroughly cleaned polyethylene capsules.

Irradiation and nuclear data

The serum capsules are irradiated for 4 h and the packed cells capsules for 6 h in the Thetis reactor of Ghent University. The flux conditions are summarized in table 1 (23).

The nuclear reactions of practical importance are listed in table 2. The only nuclear interference is due to the fast neutron treshold reaction ^{56}Fe(n · p)^{56}Mn[1]). As the iron content in serum is approximately 1.20 μg/ml, the interference is low at the flux conditions of site 1 (≦ 3%). Because of the high iron content of packed cells (approximately 1 mg/g wet weight) this interference is obvious under these irradition conditions. Therefore the samples were irradiated at a better thermalised reactor position, site 16, where the maximum interference is approximately 7%. A correction is applied based on the separately determined iron content of serum and packed cells.

The flux and spectrum of the reactor being stable, a copper wire co-irradiated with the samples was used as a neutron flux monitor.

Chemical separations

Carrier solution (10 μg Cu, 5 μg Mn and 5 μg Zn) is added after irradiation. The organic material is mineralised by wet-ashing with a 1:1 mixture of 700 g/kg perchloric acid and 14 mol/l nitric acid by heating on a hot plate. The residue is transferred with 10 ml of 1 mol/l perchloric acid into a separatory funnel. 10 ml of a saturated hydrazine sulfate solution are added in order to obtain a quantitative reduction of manganese to the bivalent state. After 2 min, 10 ml of 0.05 mol/l potassium biphthalate buffer are added. Then the solution is adjusted to pH 2 with 14 mol/l ammonia and Cu is extracted with 2 successive 10 ml portions 0.05 mol/l oxine in chloroform (25). Thereafter the pH is adjusted to 8.0 with 14 mol/l ammonia. A temporary flocculation, which appears at

[1]) n = neutron, p = proton.

Tab. 1

Flux in the irradiation positions for serum and packed cells (Thetis reactor, University of Ghent). Fluxes are given as $n \cdot cm^{-2} \cdot s^{-1}$

Material Site irradiation	Thermal flux	Epithermal flux	Fast flux	Thermal/fast flux
Serum 1	$1.10 \cdot 10^{12}$	$0.0435 \cdot 10^{12}$	$0.178 \cdot 10^{12}$	6.18
Packed cells 16	$1.69 \cdot 10^{11}$	$0.0113 \cdot 10^{11}$	$0.0187 \cdot 10^{11}$	90.4

Tab. 2

Nuclear reactions of practical importance in this study. σ_{th} = thermal activation cross section. I_0 = resonance integral. $\bar{\sigma}_f$ = fast neutron activation cross section

Nuclear reaction	% isotopic abundance stable form	σth (barn)	I_0 (barn) (24)	$\bar{\sigma}$f (barn)	Half-life (h)
$^{55}Mn(n, \gamma)^{56}Mn$	100	13.3	13.8		2.582
$^{63}Cu(n, \gamma)^{64}Cu$	69.17	4.7	6.1		12.75
$^{68}Zn(n, \gamma)^{69m}Zn$	18.57	0.075	0.23		13.7
$^{23}Na(n, \gamma)^{24}Na$	100	0.53	0.35		15
$^{37}Cl(n, \gamma)^{38}Cl$	24.47	0.435	0.213		0.622
$^{56}Fe(n \cdot p)^{56}Mn$	0.916			$0.97 \cdot 10^{-3}$	2.582

this stage, disappears during the subsequent extraction. Mn and Zn are extracted with two 10 ml portions of 0.05 mol/l oxine in chloroform. Since the activity of ^{24}Na is overwhelming in comparison with the ^{56}Mn, ^{64}Cu and ^{69m}Zn activities, traces of the aqueous phase due to incomplete separation cause a serious interference. ^{24}Na however is sufficiently eliminated by washing the organic phases with aqueous solutions of pH 2 and pH 8 respectively.

Radioactive measurements

The measurements are carried out with the following equipment:

1. NaI (Tl)detector, 3″ × 3″, 36.7 ml well type, resolution 10% for the ^{137}Cs line.
2. Intertechnique SA 40 B multichannel analyser, RG 23 magnetic tape unit and HC 20 chronometer.
3. Baird Atomic Sample Changer connected to the SA 40 B.

The sample changer is first loaded with the vials containing ^{56}Mn as this is the shortest lived species. Counting is started 3 h after the end of the irradiation.

For counting ^{56}Mn, ^{69m}Zn and ^{64}Cu respective photopeaks at 846.9 keV, 438.7 keV and 511 keV are chosen. As already mentionned ^{24}Na activity cannot be neglected. Mixed gamma-ray spectrometry was applied choosing respectively 2 (pH 2 phase) and 4 (pH 8 phase) energy regions and solving the equations for respectively 1 and 2 unknowns, i. e. ^{64}Cu with ^{24}Na interference and ^{56}Mn and ^{69m}Zn with ^{64}Cu and ^{24}Na interferences. The net activities are normalised against the copper monitor wire.

Typical spectra with the energy regions chosen are shown in Figures 1a and 1b.

Results

The chemical procedure was tested with tracer amounts of ^{56}Mn, ^{64}Cu, ^{69m}Zn and ^{24}Na. The recoveries are recorded in table 3.

The reproducibility of the method was tested in 4 sera (table 4).

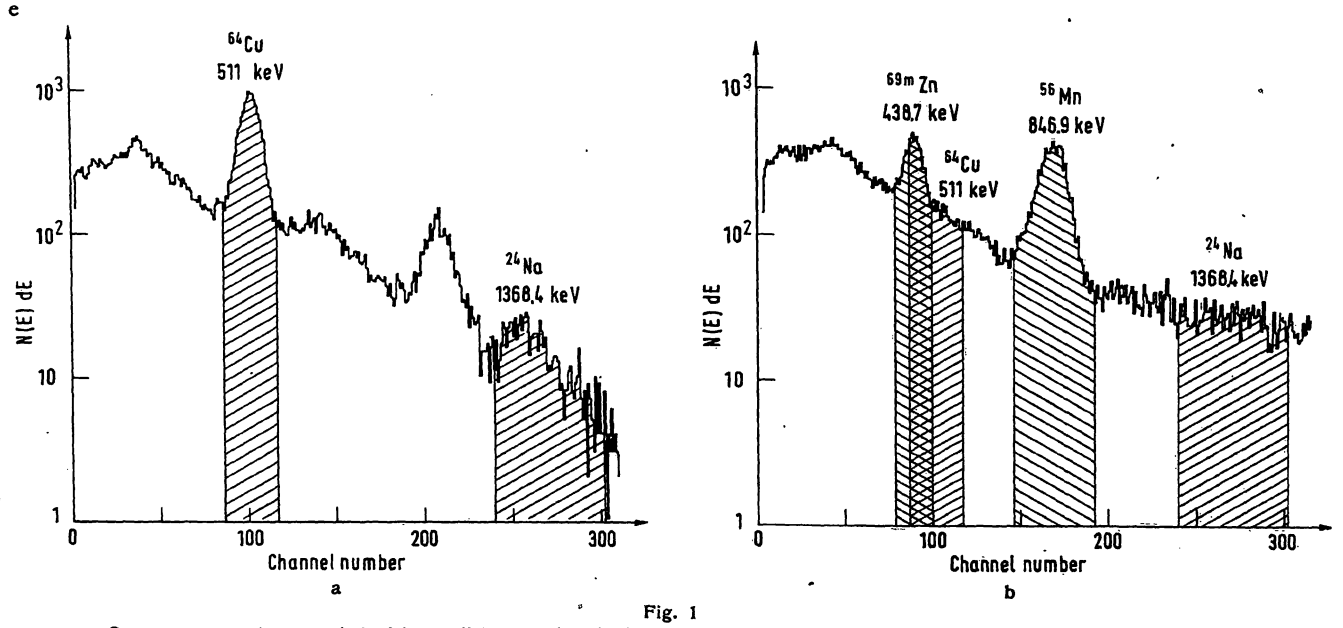

Fig. 1

Gamma-ray spectra recorded with a well-type sodium iodide (thallium activated) detector coupled to a 400 channel analyser
a) Irradiated packed cells. Wet-ashed. Extraction with oxine in chloroform. Phase at pH 2
b) Irradiated packed cells. Wet-ashed. Extraction with oxine in chloroform. Phase at pH 8

Tab. 3

Recovery of added Cu, Mn and Zn

Element	Recovery [%] pH = 2.0 mean	pH = 2.0 range	pH = 8.0 mean	pH = 8.0 range
Cu	99.8	98.7—100.8		
Mn	0.03	0.0—0.05	99.5	98.2—101.0
Zn	0.50	0.0—0.70	98.4	96.2—100.9
Na		~0.02		~0.06

The results obtained for serum and packed blood cells of 20 hospitalized patients are listed in table 5. Two samples of blood were collected from patients 1—19 and handled separately. Manganese, copper and zinc were determined in each sample. The higher manganese

Tab. 4

Reproducibility of the analytical procedure. Determinations in serum of four individuals. Manganese is given as microgram/l serum, copper and zinc as milligram/l serum

Subject	Mn (µg/l)	Cu (mg/l)	Zn (mg/l)
I			
Mean (n = 6)	0.734	1.03	1.01
S. D.	0.051	0.047	0.080
II			
Mean (n = 6)	0.640	1.36	1.00
S. D.	0.060	0.020	0.069
III			
Mean (n = 6)	0.458	1.26	0.928
S. D.	0.032	0.050	0.069
IV			
Mean (n = 6)	0.524	1.14	0.772
S. D.	0.051	0.018	0.032

Tab. 5

Values of manganese, copper and zinc in human serum and packed cells. Of each subject two samples (a and b) or two samples at an interval of two weeks (a, b and c, d) are analysed
Manganese is given as nanogram/ml serum or as nanogram/g packed cells, wet weight
Copper and zinc as microgram/ml serum or microgram/g packed cells wet weight

Subject No.	Age	Sex	Diagnosis	Sample	Mn Serum ng/ml	Mn Packed cells ng/g	Cu Serum µg/ml	Cu Packed cells µg/g	Zn Serum µg/ml	Zn Packed cells µg/g
1	51	♀	Detachment of the retina	a	0.56	13.6	0.93	0.56	0.72	9.9
				b	0.58	14.1	0.93	0.56	0.65	9.8
2	23	♀	Keratitis	a	0.64	15.3	1.54	0.69	0.79	9.6
				b	0.44	15.5	1.63	0.66	0.86	9.4
3	20	♂	Ocular injury	a	0.64	13.8	0.84	0.65	1.14	9.8
				b	0.57	13.7	0.89	0.68	1.10	9.9
4	51	♂	Detachment of the retina	a	0.58	13.1	1.01	0.70	0.90	11.4
				b	0.51	12.7	1.06	0.72	1.05	11.5
5	54	♀	Detachment of the retina	a	0.60	13.6	0.70	0.61	0.78	11.4
				b	0.60	13.8	0.85	0.62	0.74	11.8
6	22	♂	Ocular injury	a	0.72	16.5	1.12	0.70	0.67	11.1
				b	0.60	16.8	1.35	0.68	0.77	11.2
7	45	♂	Detachment of the retina	a	0.44	10.1	1.01	0.61	0.97	10.9
				b	0.49	9.0	1.02	0.64	0.87	10.8
8	32	♂	Detachment of the retina	a	0.57	13.4	0.73	0.73	1.04	11.8
				b	0.70	13.3	0.89	0.69	1.23	11.2
9	54	♂	Ocular injury	a	0.65	12.6	1.00	0.65	1.01	11.4
				b	0.81	13.4	1.18	0.68	1.09	11.2
10	19	♀	Detachment of the retina	a	0.54	17.0	1.01	0.66	0.88	9.7
				b	0.55	17.8	1.03	0.65	0.73	9.4
11	52	♂	Detachment of the retina	a	0.64	13.3	1.01	0.64	1.05	10.1
				b	0.78	13.7	0.99	0.65	0.98	10.2
12	17	♀	Keratoconus	a	1.02	15.7	0.83	0.60	0.90	11.7
				b	1.06	15.2	1.00	0.60	1.07	11.0
13	22	♀	Neurosis	a	0.57	14.7	1.41	0.70	0.97	9.8
				b	0.62	13.7	1.39	0.69	0.90	10.0
14	42	♂	Ocular injury	a	0.61	12.6	1.35	0.62	1.11	10.8
				b	0.55	12.8	1.36	0.66	0.98	10.4
15	16	♀	Ocular injury	a	0.45	15.7	1.26	0.69	0.88	10.8
				b	0.46	16.6	1.29	0.71	0.93	11.2
16	27	♀	Detachment of the retina	a	0.50	13.3	1.13	0.72	0.78	9.5
				b	0.50	13.3	1.15	0.76	0.74	9.5
17	66	♀	Keratitis	a	0.46	17.6	0.95	0.77	0.84	10.2
				b	0.56	19.0	0.96	0.73	0.89	10.4
18	62	♂	Cataract	a	0.76	20.2	1.16	0.82	0.74	11.6
				b	0.63	20.8	1.15	0.77	0.72	11.5
19	29	♂	Ocular injury	a	0.45	16.2	1.12	0.80	0.83	11.1
				b	0.61	15.1	1.09	0.68	0.78	10.0
20	27	♂	Keratoconus	a	1.02	15.2	1.30	0.71	0.96	10.2
				b	0.99	14.7	1.26	0.70	0.93	10.7
				c	1.01	14.6	1.04	0.69	1.03	10.3
				d	1.02	15.8	1.05	0.68	1.02	10.2
			Range from		0.44	9.0	0.70	0.56	0.65	9.4
			to		1.06	20.8	1.63	0.82	1.23	11.8
			Mean		0.64	14.7	1.09	0.68	0.91	10.6
			Standard deviation		0.18	2.3	0.21	0.06	0.14	0.8

concentrations of case 20 (a and b) was checked in samples taken two weeks later (c and d).

Discussion

Reliable results for copper and zinc in serum have been available for many years. Their relatively high concentration is easily determined by many analytical techniques. Adaptions have also been described for other organic material (26). Radioactivation analysis of manganese has been reported by many authors (15, 17, 18, 20, 21, 27, 28, 29), but most probably the only reliable concentration for serum manganese is that reported by COTZIAS (16) in 1966. The very low concentration represents a major technical problem: extreme precautions have to be taken to avoid contamination (16, 21, 22).

The reported copper and zinc concentrations in serum are in good agreement with the results obtained by other investigators (3, 5, 11, 12, 13, 30—37). The serum manganese concentrations are comparable to the values obtained by COTZIAS (16). In our series two relatively high values were found. In both cases, 12 and 20, the values were obtained in duplicate; in case 20 there was close agreement between samples taken at an interval of two weeks. The concentrations obtained show a relatively small range (table 5). This also corresponds to the findings of COTZIAS (16) but is in striking contrast with the data of MERTZ et al. (19). In this latter case, sample contamination could be the explanation.

The values of manganese, copper and zinc in packed blood cells also vary within narrow limits. In the literature data are rather scarce (5, 20, 35).

For manganese the ratio packed cells/serum is 24, for zinc 12, whereas the serum concentration of copper is greater than the intracellular (0.64).

As many speculations concerning the biological functions of manganese are based on concentrations that are obviously too high, it may be possible that much earlier work needs revision or confirmation. The available data concerning copper and zinc are not so open to doubt, as earlier determinations seem to have given reliable results.

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